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Data Sheet

DOT1L Chemiluminescent Assay Kit

Catalog #52202
Size: 96 reactions

DESCRIPTION: The *DOT1L Chemiluminescent Assay Kit* is designed to measure DOT1L activity for screening and profiling applications. The *DOT1L Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well plate, DOT1L substrate, primary antibody against methylated lysine residue of Histone H3, secondary HRP-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, and purified DOT1L for 100 enzyme reactions. The key to the *DOT1L Chemiluminescent Assay Kit* is a highly specific antibody that recognizes methylated K79 residue of H3. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme for two hours. Next, primary antibody is added. Finally, the strips are treated with an HRP-labeled secondary antibody followed by addition of HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51005	DOT1L	50 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120	100 µM S-adenosylmethionine	250 µl	-80°C	
	DOT1L substrate	250 µl	-80°C	
52140Y	Primary Antibody 25	12.5 µl	-80°C	
52131H	Secondary HRP-labeled Antibody 2	10 µl	-80°C	
52180	4x HMT Assay Buffer 3*	3 ml	-20°C	
52100	Blocking Buffer 4	50 ml	+4°C	
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp	
	ELISA ECL substrate B (brown bottle)	6 ml	Room Temp	
79837	96-well strip plate	1 plate		

* Add 30 µl of 0.5 M DTT before use

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
TBST buffer (1x Tris-buffered saline, pH 8.0, containing 0.05% Tween-20)
Luminometer or microplate reader capable of reading chemiluminescence
Rotating or rocker platform
0.5 M DTT

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

STABILITY: One year from date of receipt when stored as directed.

REFERENCES:
1. Min, J., *et al. Cell* 2003; **112:**711-723.
2. Daigle, S.R., *et al. Cancer Cell* 2011, **20**:53-65.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 150 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the plate onto clean paper towels to remove liquid.

2) Thaw **S-adenosylmethionine** on ice. Upon first thaw, briefly spin tube containing **S-adenosylmethionine** to recover full content of the tube. Aliquot **S-adenosylmethionine** into single use aliquots. Store remaining **S-adenosylmethionine** in aliquots at -80ºC immediately. *Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

3) Add 30 µl of 0.5 M DTT to 4x HMT Assay Buffer 3. Prepare the master mixture: N wells × (7.5 µl **4x HMT Assay Buffer 3** + 2.5 µl **100 µM S-adenosylmethionine** + 2.5 µl **DOT1L substrate** + 12.5 µl distilled water)

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	Blank	Substrate Control	Positive Control	Test Inhibitor
4x HMT assay buffer 3	7.5 µl	7.5 µl	7.5 µl	7.5 µl
100 µM S-adenosylmethionine	2.5 µl	2.5 µl	2.5 µl	2.5 µl
DOT1L substrate	2.5 µl	–	2.5 µl	2.5 µl
H_2O	12.5 µl	15 µl	12.5 µl	12.5 µl
Test Inhibitor/Activator	–	–	–	5 µl
10% DMSO in Water (inhibitor buffer)	5 µl	5 µl	5 µl	–
1x HMT assay buffer 3	20 µl	–	–	–
DOT1L (25 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

4) Add 25 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the "Substrate Control", add 7.5 µl **4x HMT Assay Buffer 3** + 2.5 µl **100 µM S-adenosylmethionine** + 15 µl distilled water

5) Add 5 µl of inhibitor solution of each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 5 µl of 10% DMSO in water (inhibitor buffer).

6) Dilute one part **4x HMT Assay Buffer 3** with 3 parts distilled water (4-fold dilution) to make **1x HMT Assay Buffer 3**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

7) Add 20 µl of **1x HMT assay buffer 3** to the well designated "Blank".

8) Thaw **DOT1L enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **DOT1L enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **DOT1L enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

9) Dilute **DOT1L enzyme** in **1x HMT assay buffer 3** at 25 ng/µl (500 ng/20 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

10) Initiate reaction by adding 20 µl of diluted **DOT1L** prepared as described above. Incubate at room temperature for two hours.

11) Wash the strips three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

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12) Add 100 µl of **Blocking Buffer 4** to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

Step 2:

1) Dilute **Primary antibody 25** 800-fold with **Blocking Buffer 4**.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash the strips three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

4) Add 100 µl of **Blocking Buffer 4** to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

Step 3:

1) Dilute **Secondary HRP-labeled antibody 2** 1,000-fold with **Blocking Buffer 4**.

2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash the strips three times with 200 µl TBST buffer. Blot dry onto clean paper towels.

4) Add 100 µl of **Blocking Buffer 4** to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

5) Just before use, mix on ice 50 µl **ELISA ECL substrate A** and 50 µl **ELISA ECL substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

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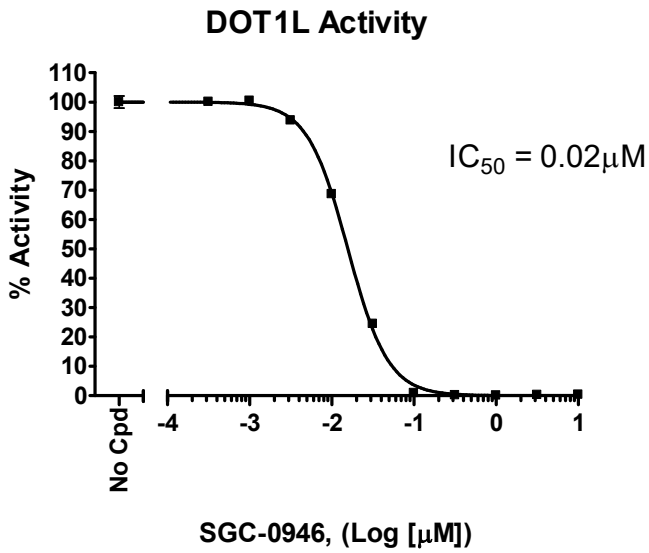
Example of Assay Results:



Inhibition of DOT1L enzyme activity by SGC 0946 (BPS Cat. #27649), measured using the *DOT1L Chemiluminescent Assay Kit*, BPS Bioscience Cat. #52202. Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

RELATED PRODUCTS

Product Name	Catalog #	Size
DOT1L	51005	50 µg
Anti–H3K79me1 polyclonal antibody	25261	50 µg
Anti–H3K79me2 polyclonal antibody	25262	50 µg
Anti–H3K79me3 polyclonal antibody	25263	50 µg
SGC 0946	27649	10 mg
EPZ5676	27625	50 mg
EPZ004777	27622	5 mg
EHMT1/GLP Chemiluminescent Assay Kit	53007	96 rxns
EZH2 Chemiluminescent Assay Kit	52009L	96 rxns
G9a Chemiluminescent Assay Kit	52001L	96 rxns
MLL1 Complex Chemiluminescent Assay Kit	53008	96 rxns
NSD1 Chemiluminescent Assay Kit	53010	96 rxns
NSD2 Chemiluminescent Assay Kit	53009	96 rxns
SETD2 Chemiluminescent Assay Kit	52060	96 rxns
SetDB1 Chemiluminescent Assay Kit	51056L	96 rxns

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	DOT1L has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh DOT1L, BPS Bioscience #51005. Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection. See section on "Reading Chemiluminescence" above.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Be sure to include blocking steps after wash steps. Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of DOT1L, BPS #51005 to create a standard curve.